BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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VOLUME XVI.

WOODS HOLE, MASS.
DECEMBER 1908 TO MAY 1909.

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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Vol. XVI. *December, 1908.* *No. 1*

BIOLOGICAL BULLETIN

REGULATION OF HARENACTIS ATTENUATA IN ALTERED ENVIRONMENT.

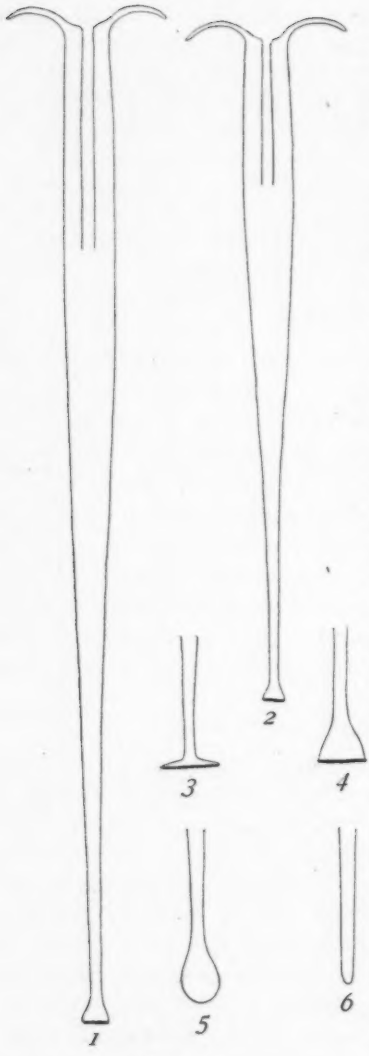
C. M. CHILD.

In the course of several months work during the autumn and winter of 1905–6 at the laboratory of the San Diego Marine Biological Association, in La Jolla, California, I had the opportunity of investigating in some detail certain of the regulatory phenomena and conditions determining them in *Harenactis attenuata* (Torrey, '02). Most of my work on this species was concerned with restitution and will be presented elsewhere. Certain of the data obtained, however, concern certain regulatory changes which occur under altered external conditions. Since these are of some interest in themselves and are not immediately related to restitution, they are presented here, apart from the other data.

I. The Usual Form and Habit.

Harenactis attenuata is found in great abundance on the tide flats of False Bay and San Diego Bay, living, as Torrey has stated (Torrey, '02, p. 384), with the column imbedded in fine sand, the axis of the body being perpendicular or nearly so to the surface. When the animal is undisturbed the tentacles, twenty-four in number, extend laterally over the surface of the sand, though very often the tentacles appear as if in two sets, twelve of them, alternating with the other twelve, extending more or less upward and sometimes curved inward, while the other twelve extend laterally and may be curved slightly downward. Often, when the body is well distended the column may protrude a short distance from the sand.

Any strong stimulus causes immediate withdrawal of the oral



FIGS. 1–6.

end from the surface, often for a distance of several inches, and usually more or less complete invagination of the disc and tentacles.

As regards size and shape, the same individual may differ greatly at different times, according to the degree of distension with water and of muscular contraction. In the condition of extreme extension and distension, the larger individuals possess approximately the shape diagrammatically outlined in Fig. 1.[1] Fig. 2 represents a shape which is perhaps nearer the average. In general I have found that the larger individuals are not only absolutely but relatively longer than the smaller. Apparently growth, at least in those later stages of development which I have observed, is chiefly in the longitudinal direction. But the length differs considerably according to external conditions. In certain regions I found the animals living in soft sand which was underlaid at a depth of 20–25 cm. by a layer of broken shells. Under these conditions the

[1] Figs. 1–6 are somewhat less than one half natural size; other figures are about three fourths natural size.

length of the animals was never much greater than the depth of the layer of sand. On the other hand, when they live in a sufficiently thick layer of sand they may attain a length of 40–50 cm. The greatest diameter of the column near the oral end is 15–25 mm. From this region the column gradually tapers to an extremely attenuated proximal portion, which differs greatly in length at different times and in different individuals.

When the animals are removed from their burrows the aboral end is usually more or less button-shaped or disc-shaped (Figs. 1–4) and firmly attached to a bit of shell, a small stone or other solid object. It is, in short, structurally and functionally a foot. In removing the animals from the sand this foot is often lost, since the attenuated region just distal to it is relatively weak. Sometimes, if the foot happens to be attached to some object near the surface — within 5–10 cm. — the attenuated region may be completely absent and the animal may possess something the shape of Fig. 10.

In some cases, however, the foot is not attached to any solid object; under these conditions it may take the forms shown in Figs. 5 and 6 or almost any form intermediate between them. No well defined adhesive surface is developed in these cases, though grains of sand may in some cases adhere to some part of the region. These marked differences, occurring under natural conditions, suggest that the differentiation of a localized adhesive foot is dependent, at least in some degree, upon contact with a solid substratum, a suggestion which is confirmed by the facts cited below. Such forms of the aboral end as those in Figs. 5 and 6 undoubtedly result from the failure of this region to come into contact with a large solid body.

The diameter of the disc is scarcely greater than the greatest diameter of the column; the tentacles of large individuals, when fully extended may reach a length of 40 mm.

The circular muscles of the body-wall are strongly developed throughout and contraction is often unequal in different parts of the body, so that marked constrictions occur. The mesenteries bear powerful longitudinal retractor muscles, which are inserted distally near the free end of the œsophagus. Contraction of these muscles invaginates the disc and tentacles (Fig. 7) and in

extreme cases reduces the length of the whole body to a small fraction of what it is when fully extended.

FIG. 7.

In the condition of extreme contraction following some violent stimulus almost all of the water is expelled from the enteron, largely through the cinclides, and the body-wall contracts until the enteric cavity is so reduced that further contraction is practically impossible on account of the bulk of the mesenteries, muscles, filaments, and gonads, if present, which now fill the enteron.

II. REGULATION FOLLOWING REMOVAL FROM SAND.

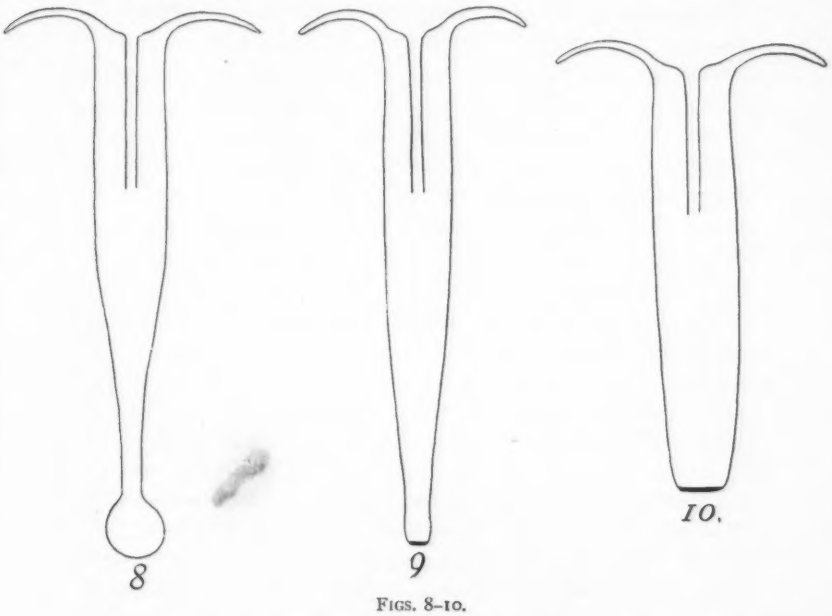
In the laboratory the animals were kept in dishes, containing water several inches in depth, but without sand. This species is exceedingly hardy and will live for months under these conditions with occasional change of water. In my experiments I kept many specimens under these conditions and without food during four and a half months, and at the end of this time they were apparently in good condition, though smaller than when collected. It is under these conditions that the changes to be described occur.

1. *The Earlier Stages of Regulation.*

As long as the animal is in its burrow in the sand the pressure upon the body-wall of the water in the enteron is supported in large measure by the walls of the burrow, not by the body-wall alone. After removal from sand the body never attains the degree of extension and distension which it may attain in the burrow. As in *Cerianthus æstuarii* (Child, '08), so here, a regulation undoubtedly occurs both as regards the amount of water usually in the enteron, and the ability of the body-wall to sustain the pressure unaided.

The effect of the altered conditions appears first chiefly in a decrease in length and frequently a slight increase in diameter of the column. Figs. 8, 9 and 10 show three common types of the shape of the body after twenty-four hours in water without sand. There is great variation in the shape of both body and foot at this time. In case the foot was originally attached to a bit of shell or other solid body it usually frees itself soon after the animal is

brought into a vessel without sand. Afterward it may become attached to the side or bottom of the vessel or may remain unattached. Very commonly when the foot is free, the aboral end of the body takes the shape of Fig. 8, though forms like Fig. 9 often appear. In some cases the attenuated posterior region is entirely absent and the animal is attached by a disc of nearly the same diameter as other parts of the body (Fig. 10), or the aboral end may be free and rounded. These various shapes are merely the visible expression of various states of contraction or extension and change more or less widely in the individual. The most interesting feature is that the body outside of its burrow never in any case, so far as my observations go, extends as completely as in the burrow.



FIGS. 8–10.

The animals in water without sand very commonly exhibit negative geotaxis in some slight degree, though never as strongly as *Cerianthus* (Loeb, '91 ; Child, '03, p. 243). No matter how firmly the foot may be attached to the substratum, the animal is

not capable of maintaining itself in an erect position in the water, so long as it retains the elongated form (*e. g.*, as in Figs. 8–10). The geotactic reaction usually manifests itself as in *Cerianthus* by the bending of the body which lies on the bottom of the dish, so that the disc lies in the horizontal plane and the axis of the body immediately below it is vertical. The reaction proceeds no further, apparently because the body is incapable of supporting itself erect. This reaction is by no means universal: very frequently it seems to be almost or wholly absent. Occasionally individuals which are strongly contracted longitudinally are found standing almost erect in the water, *i. e.*, the body-wall is capable under these conditions of supporting itself in the water.

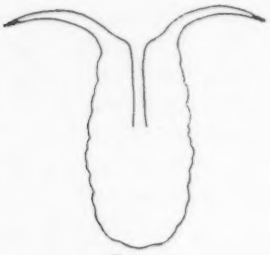


FIG. 11.

FIG. 12.

The decrease in average length continues from day to day in water without sand, though of course with many fluctuations, as the animal contracts or extends. After a week or two under these conditions the animals usually resemble Figs. 11 and 12 in shape. If the foot is attached, the aboral end is usually a flat disc as in Fig. 12, if it is free, the body is usually a more or less rounded sac without any visible demarcation of the foot-region (Fig. 11). The attached individuals usually stand more or less erect in water, and the unattached lie on their sides. In most cases the free individuals lying on their sides show little or no reaction to gravity, but the absence of the visible reaction is probably due merely to the fact that the stimulus is not sufficient to induce bending of the column in its shortened condition. If these individuals happen to become attached they often become erect.

The body-wall usually shows numerous transverse wrinkles and folds at this stage of regulation, but these gradually disappear with time.

2. *The Later Stages of Regulation.*

Any distinction between the earlier and later stages of this regulation must be more or less arbitrary. The important point, however, is that what is at first merely a condition of partial contraction and capable of rapid alteration under changed conditions, becomes, in consequence of partial atrophy of certain parts and other changes, a relatively permanent form. The transition from the one condition to the other is of course gradual and proceeds at different rates in different parts.

The first directly visible indication of atrophy occurs at the tips of the tentacles. In *Cerianthus* (Child '04*d*, '05) such atrophy of the tips of the tentacles occurs when the internal pressure due to water in the enteron decreases, and as will appear elsewhere, the same process occurs in *Harenactis* under similar conditions. In the present case the tentacle-atrophy is undoubtedly a consequence of the decreased internal water pressure which follows removal from the burrow. Usually the tentacles underwent reduction to about half their original length during the four and a half months of the experiments, though individual differences were of course considerable.

As noted above, the transverse folds and wrinkles of the body-wall gradually disappear. Extensive regulatory changes occur throughout the body-wall. In *Cerianthus* regions of the body-wall which are sharply folded or contracted for any considerable time undergo more or less atrophy (Child, '05, '08) while on the other hand stretched regions grow. The present case is similar: undoubtedly more or less atrophy occurs throughout the body-wall in consequence of the decreased distension, but the atrophy is greatest in those regions where the conditions depart most widely from the original conditions of distension, viz., in the folds. The tissue of these parts is not functioning or is functioning only in slight degree, and its substance probably undergoes resorption in consequence of the demand of other more actively functioning parts for nutrition.

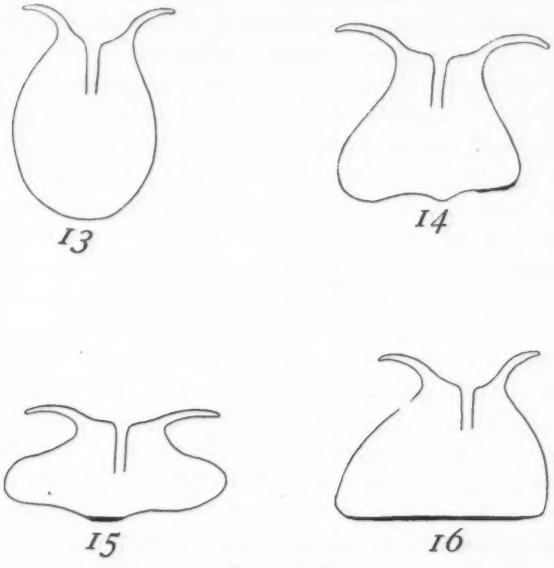
Another marked though gradual change which occurs is in the longitudinal retractor muscles. Contraction and invagination of the oral end is usually rapid and considerable in amount in animals newly removed from their burrows, but as the length of the body decreases this reaction becomes less and less marked. Animals in the condition of Figs. 11 and 12 are usually incapable of complete invagination of the disc, no matter how powerful the stimulus. Usually invagination does not include more than the basal half or perhaps two thirds of the tentacles in such cases, though originally the disc and tentacles could be withdrawn far into the body.

Two factors are probably concerned in this decrease of the power of invagination of the oral end: in the first place, the retractor muscles are undoubtedly undergoing atrophy from disuse, since as the body decreases in length their functional activity is much reduced; and secondly, the enteron is much smaller than it was originally and, as can readily be shown by opening one of these reduced specimens, is almost wholly occupied by the mesenteries with their muscles and filaments, and the gonads, if they are present, leaving but little space for water. Consequently when contraction occurs, decrease in size can occur only to the extent that water is expelled. In these specimens, after the expulsion of the small amount of water present, the body is essentially a sac packed full of tissues and no further contraction is possible unless parts of these are extruded, which does not commonly occur in *Harenactis*.

Some of the different individual shapes after four and a half months of life outside of a burrow are shown diagrammatically in Figs. 13–16. Fig. 13 represents a type very common in my experiments. These specimens have apparently lost all power to attach themselves and the aboral end shows no trace of a distinct foot-region. The whole body is merely a rounded sac with but little power of elongation or of contraction, and invagination of the oral end does not proceed beyond a slightly decreased diameter and increased concavity of the disc. Animals of this shape occur chiefly among those whose attachment to the bottom of the dish has often been disturbed, *e. g.*, by changing the water or by examination for other purposes. I am inclined to believe that

many of these individuals have completely lost the power of attaching themselves, though it is possible that the sac-like form of the body is responsible for their failure to attach, since the aboral region usually does not come into contact with the substratum.

Figs. 14 and 15 show other cases, in which the decrease in length is still greater, but in which the aboral end is more flattened and still retains the power of attachment. These cases illustrate the conditions usually found when the attachment to the



FIGS. 13-16.

substratum is less frequently disturbed. Although a flattened aboral region of greater diameter than any other part of the body is present, attachment does not always occur over the whole surface; apparently, however, any part of the surface is capable of serving as the organ of attachment, the animals being attached in some cases by a small region in the middle of the flattened surface (Fig. 15), and in other cases by some part or parts of its margin (Fig. 14). In fact the region of actual attachment may differ at different times in the same individual.

The form that is in some respects of greatest interest is shown in Fig. 16. In such cases the body is usually attached over more or less of the flattened aboral surface, though occasionally such forms appear without actual attachment, provided the body is broad enough to stand upright on its aboral end. Apparently the mere contact without attachment is sufficient to bring about more or less flattening of the aboral end. In these flattened forms the power of invagination of the oral end is almost entirely absent. Violent stimulation may bring about a slight temporary increase in the flattening, with perhaps some depression of the oral region which does not amount to actual invagination.

The interesting feature in these cases is that the originally greatly elongated, attenuated body has acquired a form almost exactly similar to that of those actinians which are normally sessile on the surfaces of rocks, etc. The ability to retract the oral end has been almost or wholly lost, but from certain observations toward the conclusion of my experiments, it seems not impossible that in the further course of regulation this power may be regained to a greater or less extent. These observations were simply that many individuals seemed to possess a greater power of retraction of the oral end after four and a half than after three and a half months. If this difference is real it may be due either to the regulation of the longitudinal muscles so that further contraction is possible, or to regulatory decrease in the bulk of mesenteries, filaments, etc., or to both factors. The volume of the retractor muscles very evidently decreases during the regulation, and it is extremely probable that the other parts, being folded and packed together so much more closely than originally, undergo more or less atrophy. In short, it appears almost certain, though the length of time covered by my experiments is insufficient for a demonstration, that *Harenactis attenuata* may, in altered environment, acquire most of the characteristics of those actinians which are normally sessile on surfaces exposed to the water.

Individuals removed from their burrows and placed upon the surface of the sand usually succeed in working their way back into it by means of the attenuated aboral end which acquires the form of Fig. 6 under these conditions. After the regulation has occurred, however, they appear to be absolutely incapable

of extending sufficiently to insert the aboral end in the sand, or else they have lost their earlier method of reaction. Whichever the case, animals like Figs. 13–16 have never been observed to enter the sand and resume their original habit, after being placed on the surface of sand or even being imbedded in it. In the latter case they usually work their way out of the sand in a few hours and lie on its surface. It seems probable, however, that if they could be kept imbedded in the sand for a considerable length of time, more or less, perhaps complete return to the original form and habit might occur. Further experiment is necessary to determine this point.

3. *The "Foot" of Harenactis.*

In the figures showing the foot, the region actually attached to the substratum is indicated by a heavier line. As the figures show at once, this region is extremely variable in extent: in Figs. 5 and 6 there is no distinct region of attachment, in Figs. 1 and 2 the region of attachment is a very small area, in Figs. 3 and 4 it is somewhat larger, in Figs. 10 and 12 it is a disc almost equal in diameter to the other regions of the column, and finally in Fig. 16 its diameter is much greater than that of any other part of the body. In Figs. 14 and 15 only a small part of the flattened aboral surface is attached, the region of attachment being in the one case in the middle and in the other on the margin. In Fig. 13 there is no trace of an adhesive region, and individuals of this sort which have been unattached during several weeks appear to be unable to attach themselves again, even if placed with the aboral end in contact, though it is probable that they might again acquire the power of attachment after a time.

The foot-region is not markedly different anatomically from the other parts of the body-wall. When an attached region is freed and examined it usually appears somewhat more transparent than other regions, but otherwise essentially similar to them. Nevertheless the region of the body within which attachment may occur is more or less sharply limited. Individuals which lie on their sides in the dishes do not become attached unless the region in contact with the dish is near the aboral end, in which case they may sometimes attach themselves.

We may conclude then that a more or less well defined aboral region of the body is usually capable of reacting to contact with solid objects by adhesion. Only those parts of this surface which are or have very lately been in actual contact are adhesive at any given time.

But the limits of the region within which reaction to contact by adhesion is possible may vary very considerably, as the observations above cited show. At the time of removal from the natural habitat the region of attachment is always limited, so far as my observations on several hundred individuals go, to a relatively very small area at the aboral end (Figs. 1–4). Sometimes after a day or two in the laboratory animals are found attached by a considerably broader area (Fig. 10), but I have never seen the slightest indication that regions still farther from the aboral pole possessed at this time the power to react in this manner, though I have often observed animals with these regions in contact. But after several months in shallow water without sand individuals like Figs. 14, 15 and 16 are of frequent occurrence. In these any portion of the broad flattened aboral region is capable of reacting to contact by adhesion and sometimes, as in Fig. 16, the whole region is involved . In Fig. 16 the radius of this foot-region is almost equal to the length of the column oral to it : if all parts of the body-wall have undergone atrophy in equal degree then the foot-region as shown in Fig. 16 consists of what was originally the aboral half more or less of the column. It is of course impossible to determine whether all parts of the body-wall have undergone atrophy in equal degree, but that point aside, it is sufficiently evident that the extent of the foot-region is very different in Figs. 1 and 16. If the regions of the body oral to the small disc of attachment in Fig. 1 were definitely specified as regions capable of attachment, we should expect to find objects adhering to them, at least occasionally, for they frequently come into contact with solid objects in the walls of the burrow. In some cases I have observed objects adhering to the oral as well as to the aboral surface of the disc of attachment, but never to regions oral to this.

In short the differences in extent of the foot-region in extreme cases like Figs. 1 and 16 show that the extent of the region

capable of the adhesive reaction may be altered very considerably by external conditions. It is probable that the adhesion of a given region increases the physiological specification of adjoining regions in the same direction, so that if these come into contact with the substratum they may adhere, and in their turn affect other adjoining regions and so on. Under the usual conditions of life other reactions, and especially the extension of the body to the surface of the sand interfere with the attachment of large regions, aborally, so that the disc of attachment usually remains very small. But when we eliminate the factor of extension and induce instead a shortening of the body, as we do in keeping the animals in water without sand, the conditions are favorable, if the aboral end is in contact at all, for bringing larger and larger areas, into contact and an extension of the actual adhesive region is the result. How far such extension might be carried it is impossible to say.

On the other hand, cases like that represented in Fig. 13 seem to indicate that the aboral region may lose the adhesive reaction if it is forced to remain unattached or is detached as often as it attaches itself. Individuals like this which had been unattached for several weeks or more failed to attach themselves even when the aboral region was brought into contact with the bottom of the dish and kept there by propping the animal up in an erect position. Such evidence is of course negative, but I believe it permits at least the conclusion that long continued absence of contact decreases the ability of the aboral end to react to contact by adhesion.

4. *Conclusion.*

The transformation within a relatively short time of the extremely elongated, burrowing type of body into the short, broad form characteristic of the sessile actinians is of interest in that it indicates the rôle which external factors play in determining the actual shape, and to a considerable extent the size relations of different parts. The elongated form is apparently impossible without the support afforded by the sand in the burrow. The chief factor in bringing about the change in form when the animals are removed from the sand is probably a change in distension of the body by water in the enteron. In animals kept with-

out sand the enteron never contains anything like the amount of water that is present when they are fully extended in the burrow. In the burrow the whole surface of the body-wall except the oral end is supported by the walls of the burrow, and so does not support the pressure of the enteric water. The oral region and the tentacles are evidently adapted to support a high pressure. Outside the burrow, however, the body-wall supports the whole pressure and one of several results is to be expected: first, distention may increase to a point where pressure of the body-wall upon its contents is so great that no more water can enter or where water passes out as rapidly as it enters; second, rupture of the body-wall may occur; third, a regulatory increase in strength or regulatory growth of the body-wall may occur; fourth, the entrance of water may be regulated by some mechanism of correlation. There is every indication that such a mechanism is present in the actinians, for distension of the body-wall beyond a certain point undoubtedly results in regulation in some degree of the quantity of water entering the body, or else in opening the outlets. Evidently such regulation occurs in *Harenactis* for the internal pressure in animals outside the burrows never approaches that which frequently exists within the burrow. The decrease in length of the body, the atrophy of the tips of the tentacles and the partial atrophy of the body-wall, the shortening of the retractor muscles and mesenteries are all very evidently consequences of the decrease in distension. As in *Cerianthus* (Child, '04, *a*, *b*, *c*, *d*, '05, '08), so here, the body-wall, the tentacles, the muscles, and mesenteries cannot maintain their form except under a certain degree of tension or stretching. When this decreases atrophy occurs to a greater or less extent and more or less change in shape may result according to conditions.

The broad foot-region of some individuals (Fig. 16) is, I believe, merely an indirect result of the change in shape which makes contact possible over a large area. Undoubtedly a broad foot-region would appear normally or occasionally in *Harenactis* if conditions existed within the burrow which permitted continued contact between a large aboral area and a solid substratum.

The transformation from the "normal" elongated form to the short, broad "sessile" shape is very evidently a regulatory reac-

tion to altered conditions. But the question as to why the elongated form is characteristic of animals in nature, *i. e.*, in burrows, has not been considered. Evidently this shape is not independent of external conditions, for when we alter the conditions the shape changes. But why should not the animal take the form of a rounded sac or a flattened disc in the sand as well as elsewhere? It is of course easy to say that the form is inherited, but besides being no explanation, this is obviously not true in this case.

In my opinion it is impossible to account for the shape of the adult *Harenactis* without considering its behavior. In the first place, the burrows of the species are almost invariably vertical or nearly so, and the behavior of the animals outside their burrows indicates a reaction to gravity. If we suppose that a young animal entering the sand has sooner or later brought its axis approximately into coincidence with the direction of gravity, muscular elongation of the body will undoubtedly occur more frequently thereafter in this direction than in any other. This of itself is without doubt a factor in determining the shape of species without hard parts. After growth in the axial direction has begun and the body is more or less elongated, there is less resistance in the sand to growth in this direction than in any other. Moreover, the aboral end is not only an organ of attachment but a burrowing organ. So long as the end comes into contact only with the soft sand, the animal tends to extend and to force the end deeper and deeper. Thus these different factors combine to bring about greater growth in the longitudinal than in other directions. As was noted above (pp. 2–3), the lengths of the animals differ very greatly according to the depth of the layer of sand in which they live. The longest individuals I have ever found were in sand which was almost free from stones or broken shell for a depth of 40–50 cm. and many of the animals living there had attained this length and were attached. But these animals were not simply more elongated than those living under other conditions, they were actually larger. The diameter was fully as great as that of the largest specimens found under other conditions and in the contracted condition it was clearly evident that the individuals from the deep sand possessed much greater

bulk than those from shallow sand. It is scarcely probable that the difference was due to difference in nutrition, for so far as it was possible to judge, the region of deep sand was much more barren of life and organic matter than the other. I think there can be no doubt that growth in the longitudinal direction is stimulated "functionally" so long as the aboral end does not come into contact with some solid surface to which it can attach.

In animals which possess no hard parts and in which muscles and supporting tissues form an important part of the body, the shape of the body, the direction of growth, and the relative size of various parts, *must* be determined, at least in large measure, by the functional conditions accompanying muscular contraction and by other mechanical factors. These factors may act either directly, as deforming factors, or indirectly through the "functional" stimulus, or they may act in both ways. Such species determine their own form in greater or less degree by their behavior. If we alter experimentally the character or energy of their reactions some more or less extensive morphological change is likely to occur. In such cases it is merely the capacity for reaction that is given in the germ, *i. e.*, inherited. The actual result in any given case depends on the internal and external conditions of development.

In the "Studies on Regulation" I have discussed the significance of certain mechanical and "functional" factors for the shape and certain other features of the structure of turbellaria, and in "Form Regulation in *Cerianthus*" for this form. The regulation of *Harenactis* in altered environment shows very clearly that similar factors are important in determining shape and other structural relations in this species, and the importance of these factors will appear still more clearly from the data of restitution.

HULL ZOÖLOGICAL LABORATORY,
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October, 1908.

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ON THE USE OF CARBON DIOXIDE IN KILLING MARINE ANIMALS.

ALFRED G. MAYER.

For several years I have been making use of sea-water charged with CO_2 for producing anæsthesia in marine animals, thus enabling one to kill them in an expanded state.

One of the soda-water siphon-bottles, sold in the market under the trade name of "Sparklet Bottles," is filled with sea-water and this is charged with CO_2 by means of the "Sparklet bulb."

This charged sea-water is then poured into a vessel containing ordinary sea-water in which the marine animals are living, and in a few moments they are completely narcotized, and may then be killed in a fully expanded state by the addition of any killing fluid.

In some instances the CO_2 should be followed by the addition of a small quantity of chloretone, but this appears to be necessary only in the case of Siphonophoræ wherein it entirely prevents the casting off of the swimming bells.

This simple method enables one to obtain excellent preparations for histological or for museum purposes of such delicate marine animals as the medusæ. It is also applicable to Crustacea, Worms, Mollusca, etc.

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EFFECTS OF REMOVING THE GERM-CELL DETERMINANTS FROM THE EGGS OF SOME CHRYSOMELID BEETLES. PRELIMINARY REPORT.[1]

R. W. HEGNER.

A number of experiments were made during the spring and summer of 1908 in order to test the conclusions reached in a previous study of the origin and early history of the germ-cells in *Calligrapha multipunctata* and several other chrysomelid beetles. These experiments suggested some interesting hypotheses which will be verified or disproved as soon as more material can be obtained. The work was begun at the Zoölogical Laboratory of the University of Wisconsin and continued at the Marine Biological Laboratory, Woods Hole, Mass.,[2] and at the Zoölogical Laboratory of the University of Michigan.

In a paper soon to be published[3] I have shown that there is an intimate connection between the primordial germ-cells and a disc of granules lying near the posterior end of the eggs of *Calligrapha multipunctata*, *C. bigsbyana*, *C. lunata* and *Leptinotarsa decemlineata*. This study led to an attempt to secure confirmatory experimental evidence. A brief account of the structure of the beetle's egg and the early history of the germ-cells is necessary for a clear understanding of the method of procedure and of the results.

The freshly laid eggs of these beetles consist of a thin peripheral layer of cytoplasm (the "Kleimhautblastem" of Weismann) and a relatively large central mass composed of yolk globules with a small amount of cytoplasm filling the inter-

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 120.

[2] The writer is indebted to the Wistar Institute of Anatomy and Biology for the use of a room at the Marine Biological Laboratory, Woods Hole, Mass., during the summer of 1908.

[3] "The Origin and Early History of the Germ-Cells in some Chrysomelid Beetles." Accepted for publication by the *Journal of Morphology*.

deutoplasmic spaces. When the egg is deposited, the germinal vesicle, has already moved to a point near the ventral surface just a trifle anterior to the center of the egg; here polar-body formation takes place. One or more male nuclei are present near the anterior end of the egg. Suspended in the "Keimhautblastem" near the posterior end of the egg is a mass of deeply staining granules which I have called the "pole-disc." These granules occupy the inner portion of the peripheral cytoplasmic layer and cover about one eighth of the area at this end of the egg (Fig. 1). Surrounding the egg are the vitelline membrane and the chorion.

After the conjugation of the sperm-nucleus with the egg-nucleus, which occurs a few hours after the eggs are laid, the

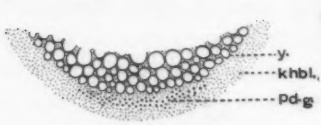

FIG. 1.

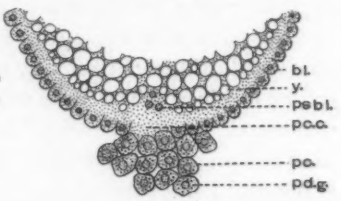

FIG. 2.

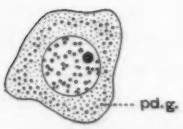

FIG. 3.

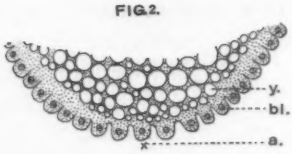

FIG. 4.

FIG. 1. A portion of the posterior end of an egg of *C. bigsbyana* in longitudinal section showing the arrangement of the pole-disc granules (*pd.g*). *y* = yolk; *khbl* = "Keimhautblastem."

FIG. 2. Longitudinal section through the posterior end of an egg of *C. bigsbyana* showing the primordial germ-cells (*pc*) containing pole-disc granules (*pd.g*). Nuclei which are prevented from forming blastoderm cells produce a pseudoblastodermic syncytium (*psbl*). The letters *pc.c* indicate the canal through which the pole-cells later migrate into the embryo. *bl* = blastoderm. *y* = yolk.

FIG. 3. A single primordial germ-cell showing pole-disc granules (*pd.g*).

FIG. 4. Longitudinal section through the posterior end of the egg of *C. lunata* described in Exp. 2*a*. The letter *a* indicates where germ-cells would be found in an uninjured egg. *bl* = blastoderm. *y* = yolk.

cleavage nuclei increase rapidly in numbers and migrate in all directions toward the periphery reaching the "Kleimhautblastem" at the expiration of eighteen hours. Those nuclei (sixteen in

number) that come in contact with the granules of the pole-disc, do not remain in the "Kleimhautblastem" and later become the centers of blastoderm cells as do the other nuclei but become completely surrounded by a thin layer of these darkly staining bodies and continue their migration until they are entirely separated from the egg. They then lie between the vitelline membrane and the blastoderm in a closely packed group (Fig. 2). Each of these sixteen cells ("pole-cells") has the appearance of the one shown in Fig. 3. They soon divide by mitosis to form thirty-two. During this division the pole-disc granules are equally distributed among the daughter cells. Another division increases the number to sixty-four. This number is reached at the end of twenty-four hours and no further divisions take place until about the time of hatching. The blastoderm cells increase rapidly in number and can be distinguished from the cells just described not only because of the absence of granules but also by the smaller size of their nuclei. No difficulty was experienced in tracing the sixty-four cells containing pole-disc granules until they became the definite germ-gland.

These phenomena give reasonable grounds for the following conclusions. All the cleavage nuclei in the eggs of the above named beetles are potentially alike until in their migration toward the periphery they reach the "Keimhautblastem." Then those which chance to encounter the granules of the pole-disc are differentiated by their environment, *i. e.*, the granules, into germ-cells; all the other cleavage products become somatic cells. The granules of the pole-disc are therefore either the germ-cell determinants or the visible sign of the germ-cell determinants. The term determinant is not used here in the Weismannian sense but simply is meant to describe the material which fixes the character of the cells.

At first an attempt was made to tie off the posterior end of freshly laid eggs by means of fine silk thread in order to remove the pole-disc. This proved impossible for in every case the pressure burst the chorion. It was later found that if the chorion is punctured with a needle at any point a small drop of the contents is forced out by the turgidity of the egg and can be removed with filter paper. The eggs of these insects are perfectly oriented

when laid. They were carefully placed with the posterior end up in small cavities in a block of paraffine. With the aid of a binocular microscope the point of a fine needle was then inserted at the center of this end, thus allowing that portion of the egg containing the pole-disc granules or primordial germ-cells to flow out. After removing this the eggs were placed in watch glasses and allowed to develop to the desired stage. Some difficulty was experienced in removing the proper amount of material and a number of the eggs either developed into shapeless masses of tissue or did not fix properly. These sources of error are in large part responsible for the failure to obtain more definite results.

I. The Effects of Removing the Pole-Disc Granules.

1. *C. multipunctata*, Exp. C.m. 1. — One egg of *C. multipunctata* was laid on April 15, 1908, and operated on while fresh. The portion removed from the posterior end was fixed on a slide and stained. It was found to contain the pole-disc granules. This egg did not hatch in the average period (5⅔ days) and so was preserved at the age of 8 days.[1] The embryo appeared to be normal in every way, but on sectioning it was found to be lacking not only in germ-cells but also in other abdominal tissues. The mid- and hind-intestines were not completely formed and other neighboring parts were likewise deficient. It is evident that the germ-cell determinants were removed by the operation and that so much of the other organ-forming substances was also removed that the embryo failed to develop the corresponding tissues.

2. *C. lunata*, Exp. C. 1. — An egg of *C. lunata* which was laid at 12 o'clock noon July 16, was operated at 2 p. m. on the same day and fixed forty-nine hours later. It was stained in toto with hæmalum, drawn and then sectioned. Externally it resembled exactly embryos of this age developed from entire eggs. Germ-cells were found in the last two abdominal segments in a position similar to that occupied by them in normal embryos. However, there were not sixty-four, as was found to be the case in embryos of *C. multipunctata*, but only thirty germ-cells could be

[1] It has often been observed that when eggs of this beetle do not hatch the embryos live for several days after the expiration of the regular hatching period.

found. It seems probable that not all of the pole-disc granules were removed in the operation and that those which were left behind determined the character of the cells which encountered them, the result being a smaller number of germ-cells.

3. *C. lunata*, Exp. C. 3. — This larva hatched at noon on July 22, exactly six days after the egg was laid. This constitutes a period eight hours longer than has been computed as the average time for eggs of this species.[1] Apparently very little if any delay in development was caused by the operation. This larva was normal in external appearance and was as active as those hatched from eggs which had not been injured. It was killed when three days old. No germ-glands could be discovered on sectioning, but as several sections were ruined by an accident no incontestible statement as to their presence or absence can be made.

4. *C. lunata*, Exp. 2*a*. — This egg was laid and operated on at 10 a. m. July 24 and was fixed at the expiration of twenty-two hours. At this age a normal egg has a group of primordial germ-cells between the vitelline membrane and the blastoderm at the posterior end (Fig. 2). No germ-cells are present in the operated egg; the blastoderm cells at the posterior pole (Fig. 4) are less numerous than at other points on the surface indicating a delayed development in this region due to the removal of the "Keimhautblastem."

5. *C. lunata*, Exp. 2*b*. — This egg was laid and operated on at the same time as the egg in Exp. 2*a*. It was preserved when thirty-six hours old. At this stage in uninjured eggs the germ-cells are present as a group near the posterior end of the ventral groove. No germ-cells were found in the operated egg but a mass of yolk and cytoplasm was discovered outside of the vitelline membrane near the posterior end.

II. The Effects of Removing the Primordial Germ-Cells.

In two experiments on stages shown in Fig. 2 eggs were punctured and the germ-cells allowed to flow out. The results indicate that all of the germ-cells were not removed in every case.

[1] For an account of the life histories of these beetles see R. W. Hegner, "Observations on the Breeding Habits of three Chrysomelid Beetles, *Calligrapha bigsbyana*, *C. multipunctata* and *C. lunata*," *Psyche*, Vol. 15, pp. 21–24.

It is the intention of the writer to repeat these operations next spring.

1. *C. lunata*, Exp. 6*b*. — An egg which was laid at 11 a. m. on July 23, was allowed to develop for twenty-five hours and then was operated on. It was preserved forty-four hours after the operation. Twenty-three germ-cells instead of sixty-four were found occupying the normal position in the embryo. Four of these twenty-three cells were in the posterior amniotic cavity while nineteen were scattered among the mesoderm cells in the last two abdominal segments. This difference in number can be accounted for by supposing that part of them were removed in the operation.

2. *C. lunata*, Exp. 6*c*. — A larva hatched from an egg which was laid and operated on at the same time as the egg in Exp. 6*a*. The larva was preserved at once. No germ-glands could be found in the sections. Several sections broke on the knife but these were from a part of the larva outside of the germ-gland region.

3. *L. decemlineata*, Exps. p. 1–7. — It is evident from the results of these experiments that either all of the primordial germ-cells were not removed from the egg during the operation or else some other cells have taken on the function of germ-cells. In one case (*L. decemlineata*, Exp. *p*. 2) it is certain that all the pole-cells were not removed. In two instances (Exps. *p*. 4 and *p*. 6) germ-glands were undoubtedly present. The method of operating therefore needs to be improved before decisive results can be secured.

III. The Effects of Removing Other Portions of the Egg.

Only one experiment was made to note the effect of removing any part of the egg other than the posterior end. The anterior end of a freshly laid egg of *C. bigsbyana* (Exp. *C. b*. 1) was punctured and a large drop of the contents removed. A normal larva hatched from this egg, passed through the larval and pupal stages and is now hibernating as an adult.

The above experiments prove that a part of an insect's egg may be removed without preventing the development of the embryo and subsequent hatching of the larva. More work must be

TABLE GIVING THE NATURE AND RESULTS OF ALL THE EXPERIMENTS.

Name.	No. of Experiment.	Age of Egg When Operated.	Operation.	Interval Between Operation and Fixation.	External Condition of Embryo or Larva.	Sections.	Germ-Cells.	Histological Condition.
C. multipunctata	C. m. 1	fresh	pole-disc removed	8 days	normal	perfect	not present	imperfect
C. lunata	C. 1	2 hours	"	49 hours	"	"	fewer than normal	perfect
"	C. 2	"	"	97 "	"	"	not found	"
"	C. 3	"	"	9 days; larva 3 days	"	several lost	"	"
"	2. a	fresh	"	22 hours	post. end altered	perfect	"	"
"	2. b	"	"	36 "	"	"	"	"
"	2. c	"	"	48 "	abnormal	"	"	abnormal
"	2. d	"	"	72 "	ruined by accident			
"	2. e	"	"	7 days	abnormal	perfect	not found	abnormal
"	5. a	"	"	24 hours	ruined by accident			
"	5. b	"	"	44 "	"			
"	5. c	"	"	68 "	abnormal	perfect	not found	abnormal
"	5. d	"	"	9 days	"	"	"	"
L. decemlineata	L. d. 1	"	"	10 "; 4 day larva	normal	"	"	normal
"	L. d. 2	"	"	72 hours	"	several lost	"	"
"	L. d. 3	"	"	6 days; 1 day larva	"	"	"	"
"	L. d. 4	"	"	48 hours	"	perfect	"	"
C. lunata	6. a	25 hours	primordial germ-cells removed	24 "	"	"	"	"
"	6. b	"	"	44 "	"	"	fewer than normal	"
"	6. c	"	"	7 days; 1 day larva	"	several lost	not found	"
L. decemlineata	p. 1	control	none		"	perfect	present	"
"	p. 2	26 hours	primordial germ-cells removed	fixed at once	"	"	fewer than normal	"
"	p. 3	"	"	16 hours	abnormal	"	not found	abnormal
"	p. 4	"	"	48 "	normal	"	present	normal
"	p. 5	"	"	72 "	abnormal	several lost	not found	abnormal
"	p. 6	"	"	104 "; 1 day larva	normal	perfect	present	normal
"	p. 7	"	"	8 days; 4 day larva	"	several lost	not found	"
C. bigsbyana	C. b. 1	fresh	ant. end removed		hibernating (Oct. 30, 1908) as an adult			

done before any final statement can be made as to the effects of removing the germ-cell determinants (pole-disc granules), but it seems probable that if all of these are taken from the egg no germ-cells will be produced. Previous researches have proven beyond a doubt that the pole-disc granules are taken up by the primordial germ-cells. It remains however for future experiments to decide whether these granules are really the germ-cell determinants or are non-essential in the differentiation of the cleavage products.

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October 30, 1908.